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The interobserver reliability of a novel qualitative point of care assay for heart-type fatty acid binding protein

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Abstract

Background

Heart-type fatty acid–binding protein (h-FABP) may help to improve the early diagnosis of acute coronary syndromes in patients presenting to the Emergency Department (ED) with chest pain. A novel qualitative point of care h-FABP lateral flow immunoassay (True Rapid, FABPulous BV) could provide results to clinicians within just 5 minutes. Given the qualitative nature of this test and prior to evaluation in a large diagnostic study, we aimed to determine inter-observer reliability when interpreted contemporaneously by staff in the ED.

Methods

In a nested prospective cohort study including adult patients with suspected cardiac chest pain, venous blood samples were tested for h-FABP (FABPulous BV) on arrival and 3 hours later. Each test result was independently interpreted by two different investigators after 5 minutes. The investigators were blinded to each other's interpretation and recorded their findings on separate case report forms. We determined interobserver reliability by calculating the Cohen's kappa score and 95% confidence intervals.

Results

A total of 43 test results (from 31 patients) were each interpreted by two independent investigators. Absolute agreement between investigators was 93.0%, with a Cohen's kappa of 0.81 (95% CI 0.6 – 1.0), indicating near perfect agreement. In total there were three (7.0%) disagreements. In each case one investigator reported a 'weak positive' result while the other interpreted the result as 'negative'.

Conclusions

These findings demonstrate the interobserver reliability of a qualitative point of care h-FABP assay. Further work must evaluate diagnostic accuracy and determine the clinical implications of the small rate of disagreement.

Background

Heart-type fatty acid binding protein (h-FABP) is abundantly present in cardiac myocytes. Its low molecular weight and cytoplasmic location mean that release is rapid after myocardial injury, which is measurable within as little as 30 minutes after onset [1, 2]. For this reason, it is an attractive biomarker for use in the early diagnosis of acute coronary syndromes (ACS) and it may be the earliest available plasma marker of acute myocardial injury [3, 4].

Among patients presenting to the Emergency Department (ED) with suspected ACS, h-FABP has been shown to be a strong independent predictor of major adverse cardiac events (MACE) within 30 days even after accounting for high sensitivity cardiac troponin, electrocardiographic (ECG) changes and clinical findings. As such, h-FABP was incorporated in the Manchester Acute Coronary Syndromes (MACS) decision rule [5]. This rule effectively risk stratifies patients following a single blood test for high-sensitivity cardiac troponin and h-FABP, taken at the time of arrival in the ED. It can be used to identify subjects who could have ACS immediately 'ruled out' (thus avoiding unnecessary hospital admission) and others in whom the diagnosis could be immediately 'ruled in'.

The MACS rule has now been validated with an automated laboratory-based h-FABP assay [6]. However, the requirement to run the h-FABP assay may present a barrier to clinical implementation, as this assay is not routinely used for other indications. The availability of a point of care (POC) assay may therefore aid clinical implementation.

There are several commercially available qualitative and quantitative POC h-FABP assays. While quantitative assays report results as continuous variables and thus provide clinicians with a 'number', disadvantages of quantitative assays include the financial cost of analyzers and the requirement for regular quality control and calibration, which is often delegated to already over-

stretched clinical staff. Qualitative assays, which provide a simple dichotomous result (positive or negative) have the advantage of removing the need for a separate analyser, thus reducing financial cost and saving staff time, although these potential benefits may depend on institution-specific factors.

Qualitative assays do, however, require interpretation, which has an element of subjectivity. An important first step in their evaluation is therefore to establish inter-observer reliability in clinical practice. If sufficient inter-observer reliability can be demonstrated, further larger studies are required to determine diagnostic accuracy.

We aimed to evaluate the inter-observer reliability a novel commercially available lateral flow immunoassay for h-FABP interpreted contemporaneously by staff in the ED.

Methods

In a prospective cohort study nested within the larger Bedside Evaluation of Sensitive Troponin (BEST) study (the objectives of which include evaluation of the diagnostic accuracy of point of care h-FABP and troponin testing), we included consenting patients who presented to the ED with suspected cardiac chest pain within 12 hours of symptom onset. Participants were selected for inclusion in this substudy by convenience sampling, dictated by the availability of two trained independent observers. Venous blood was drawn on arrival and 3 hours later (without additive). Fresh whole blood samples were then immediately tested for h-FABP using the FABPulous h-FABP True Rapid Test (FABPulous BV, Maastricht, Netherlands; calibrated to provide a positive result at 4ng/ml) according to manufacturer's instructions. This is a lateral flow immunoassay (LIA) combined with an integrated, one-step plasma filtration device that delivers whole blood into a correctly and consistently diluted plasma [7].

After 5 minutes, two independent observers including clinical research nurses, emergency physicians and a medical technologist interpreted each test. Each investigator had received a two-hour training

session on how to perform and interpret the test results and had the same training and background as staff who would be expected to interpret point of care test results in routine clinical practice. The observers were both blinded to each other's interpretation and recorded their interpretation on separate case report forms. The investigators interpreted the result from the same test without delay. Interpretation was timed using a stop clock to ensure protocol adherence and a maximum delay of 2 minutes was permitted between the two interpretations. The study was approved by the Research Ethics Committee (reference 14/NW/1344).

We determined interobserver reliability by calculating the kappa (κ) score and 95% confidence intervals using SPSS version 20.0. The kappa (κ) score was further categorised according to the classification of Landis and Koch [8] (Table 1). Sample size was calculated according to guidelines for reliability studies [9]. To demonstrate a κ of 0.8 with the minimal acceptable κ set at 0.6 and, setting the alpha at 0.05 and beta at 0.2, would require 39 patients to be assessed by two independent observers.

Table 1 Interpretation of Cohen's kappa classification by Landis and Koch is often used to judge the strength of agreement for κ [8]

Value of kappa	**Level of agreement**
<0	Poor agreement
0.0 – 0.2	Slight agreement
0.2 – 0.4	Fair agreement

0.4 – 0.6	Moderate agreement
0.6 – 0.8	Substantial agreement

0.8 – 1.0	Almost perfect agreement

Results

The study was conducted from 2nd April to 8th May 2016. A total of 43 test results were interpreted by each of two independent investigators for this study (26 drawn on arrival and 17 at 3 hours). The tests were drawn from a total of 31 patients (12 of whom had tests from both arrival and 3 hours later interpreted by two investigators). Basic characteristics of the study population are shown in Table 2. A total of 10 observers interpreted h-FABP results, including four doctors (emergency physicians), five nurses and one medical technologist. A total of 21 (24.4%) test results were positive for h-FABP.

Table 2 Basic characteristics

Study participants (n=31)		
Age, mean (standard deviation)		60 ± 12
Male sex, n (%)		15 (48.4)
Female sex, n (%)		16 (51.6)
Hypertension, n (%)		14 (45.2)
Hyperlipidaemia, n (%)		6 (19.4)
Diabetes mellitus, n (%)		1 (3.2)
Current smoker, n (%)		5 (16.1)
Previous ischaemic heart disease, n (%)		3 (9.7)
Renal impairment, n (%)		1 (3.2)
Clinicians interpreting test results		
Background of clinician interpreting test result, n (%)	Emergency physician	47 (54.7)
	Nurse (Clinical Research	18 (20.9)

Absolute agreement between investigators was 93.0%, with a kappa score of 0.81 (95% CI 0.6 to

	Nurse)	
	Medical technologist	21 (24.4)

1.0), indicating near perfect agreement between investigators. In total there were three (7.0%) disagreements in test result interpretation. Each of these disagreements appears to have arisen because one observer noted that a result was 'weakly positive' while the other rated the result as 'negative'. In one patient, the discrepancy was noted in the admission blood sample although both observers recorded the result as 'positive' when the 3-hour sample was tested. The other two patients had concordant results for the admission blood samples (both positive in one patient; both negative in the other) but discrepancies were noted at 3 hours. As tests were run in whole blood, we could not directly evaluate the impact of haemolysis in this study. No tests in this study were reported as uninterpretable.

Discussion

In this work, we have demonstrated that the results of a novel lateral flow immunoassay for h-FABP can be interpreted in clinical settings by staff with various backgrounds with a high degree of interobserver reliability. A kappa score of 0.81 demonstrates near perfect agreement and the lower bound of the 95% confidence interval is >0.6 indicating at least substantial agreement. These findings support the use of this test in clinical practice.

We did, however, note three discrepancies from our 43 observations, indicating discrepant results in 7% of cases. If results were to be wrongly interpreted as 'negative', this could lead to missed diagnosis of ACS. However, the test has been calibrated to provide positive results at 4ng/ml, which is lower than the reported 99th percentile (5.6ng/ml) of quantitative measurements[10]. This has the

effect of introducing a 'safety margin' to avoid genuine false negative results. The impact, however, should be robustly evaluated in diagnostic studies prior to implementation.

Although h-FABP is not currently widely used, it may help to facilitate early 'rule in' and 'rule out' of acute coronary syndromes in the ED, which would help to reduce unnecessary hospital admissions and identify the highest risk patients at the earliest possible opportunity. H-FABP is incorporated in the MACS decision rule, which has been shown to identify over one quarter of patients as eligible for immediate discharge from the ED with no missed AMIs and a low incidence of major adverse cardiac events within 30 days [6]. Although the MACS rule has been validated using an automated immunoassay for h-FABP, which can be run using commercial laboratory analysers, the requirement to run an assay for an additional biomarker may present a barrier to clinical implementation. A point of care test may facilitate clinical implementation. The lateral flow immunoassay described here is a simple and inexpensive technology. It requires only a drop of blood and produces results with a turnaround time of just 5 minutes. This novel assay may therefore enable the MACS rule to be used in other settings such as the pre-hospital environment or the community.

Our study does have some limitations. While our approach was pragmatic, the observers in this study had different levels of clinical experience. Each investigator received a standard 2 hour training session prior to the study commencing but was previously naïve to the use of this assay. It is possible that interobserver reliability will improve over time as staff members become more experienced in its use. Our study cannot evaluate this. It is also important to note that this study did not aim to evaluate diagnostic accuracy, which must also be established before the assay can be used in clinical settings. This is the objective of our ongoing work and will require a larger sample size. Prior to investing in such work, it was important to evaluate interobserver reliability. Had we

identified suboptimal reliability, it would have been unlikely that clinical implementation was possible making further evaluation undesirable.

Conclusion

We have demonstrated that the novel lateral flow immunoassay for h-FABP from FABPulous has near perfect interobserver reliability when used in a clinical setting in a real world ED. Future work must now establish diagnostic accuracy prior to clinical implementation.

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